

Two Novel Alkaloids with a Unique Fused Hexacyclic Skeleton from *Daphniphyllum subverticillatum*

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Received June 11, 2003

Two novel major alkaloids, deoxycalyciphylline B (**1**) and deoxyisocalyciphylline B (**2**) with a unique fused hexacyclic skeleton, together with a quite recently reported alkaloid calyciphylline B (**3**), were isolated from the stem of *Daphniphyllum subverticillatum*. Their structures were established by spectral methods and chemical evidence, especially 2D NMR techniques. The structure of **1** was further confirmed by a single-crystal X-ray diffraction determination.

Introduction

Daphniphyllum alkaloids¹ isolated from the oriental genus *Daphniphyllum* are of structurally diversified types and possess highly complex polycyclic structures. The diversified structures of *Daphniphyllum* alkaloids were classified into six main types of nitrogen heterocyclic skeletons. Recently, Kobayashi, Morita, Jossang, and co-workers reported a number of additional new tapes of *Daphniphyllum* alkaloids.^{1a–j} The biogenetic synthesis² and transformations³ of some *Daphniphyllum* alkaloids were well demonstrated, and the total syntheses⁴ of several alkaloids were also achieved. A few *Daphniphyllum* alkaloids showed remarkable cytotoxic activities against several human tumor cell lines.^{1b,c} The genus of

Daphniphyllum (Daphniphyllaceae), comprising about 30 species, is endemically distributed over the southeast of Asia. Among them, 10 species grow in the south China,⁵ some of which, such as *D. calycinum*, *D. macropodum*, and *D. oldhami*, are used in traditional Chinese medicine for the treatment of asthma,⁶ cough, rheumatism, inflammation, fever, and snakebite.⁷

Daphniphyllum subverticillatum Merr. has not previously been investigated chemically. In the current project, two novel alkaloids, deoxycalyciphylline B (**1**) and deoxyisocalyciphylline B (**2**) representing a unique fused hexacyclic skeleton, together with one quite recently isolated known alkaloid calyciphylline B (**3**),^{1a} were isolated from the stem of *Daphniphyllum subverticillatum*. The structures of these compounds were elucidated by spectroscopic methods and chemical evidence, especially 2D NMR techniques. The structure of deoxycalyciphylline B (**1**) was confirmed by single-crystal X-ray diffraction. The unique structures of compounds (**1–3**) can be rationalized biogenetically as showed in the Scheme 1. A new artificial alkaloid, namely, isocalyciphylline B (**4**), was also obtained by oxidation of **2** with *m*-CPBA (*m*-chloroperbenzoic acid). Herein, we report the isolation and structural elucidation of these alkaloids (**1–3**) from the stem of *D. subverticillatum*.

Results and Discussion

Deoxycalyciphylline B (**1**) was obtained as an optically active ($[\alpha]_D^{20} -96.0^\circ$) quadrate crystal (in acetone). The HR-EIMS of **1** exhibiting the molecular ion at *m/z* 341.2346 established the molecular formula C₂₂H₃₄NO₂

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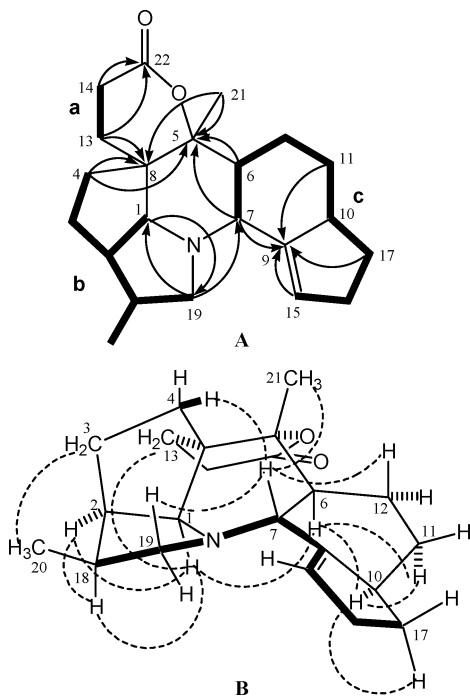


FIGURE 1. A: (---) ^1H - ^1H COSY of **1**; (—) selected HMBC correlations of **1** (H \rightarrow C). B: (---) Key NOESY correlations of **1**.

carbons and two sp^3 methyls, were resolved in the ^{13}C NMR and DEPT spectra. One carbonyl group and the only double bond accounted for two degrees of unsaturation; the remaining six degrees of unsaturation were assumed for the presence of a hexacyclic system in compound **1**.

Three structural fragments **a** (C-13 to C-14), **b** (C-1 to C-4 and C-18 to C-20), and **c** (C-6 to C-7, C-10 to C-12, and C-15 to C-17) drawn with bold bonds were established by using a combination of 2D NMR spectra (HMQC, ^1H - ^1H COSY and HMBC) (Figure 1A). The overlapping proton signals made it uncertain to obtain the structural fragments **a**–**c** only from the HMQC and ^1H - ^1H COSY. The extensive analysis of HMBC correlations was thus applied to confirm the assignments for the structural fragments **a**–**c**. The linkage of **a**, **b**, and **c** was finally made by the HMBC experiment, in which the “loose ends” resulting from the insertion of the oxygen, nitrogen, and quaternary carbons of C-5, C-8, C-9, and C-22 into the fragments could be fully connected. In the HMBC, the quaternary carbon signal at δ 172.9 was allocated to C-22 by the strong correlations between C-22 and H₂-14 at δ 2.46 (2H, m); the quaternary carbon signal of C-8 (δ 52.1) was correlated with H₂-4 (δ 1.77, 1.35) and H₂-13 (2H, δ 1.62) to connect fragments **a** and **b**. Two methines (CH-7, δ_{C} 56.8, δ_{H} 3.03; CH-1, δ_{C} 69.4, δ_{H} 3.64) and one methylene (CH₂-19, δ_{C} 59.2, δ_{H} 3.12, 2.02) attributable to those attached to the nitrogen were indicative of the connectivity between the partial structures **b** and **c** by the nitrogen atom, and this was confirmed by the HMBC correlations between the atom pairs of H-19 β /C-1, H-1/C-19, and H-19 β /C-7. The linkages of the C-9 to the C-7 and C-15 were made by the HMBC correlations between H-7 and C-9, and between H-15 and C-9. Although the correlation between H-10 and C-9 (J^{β}) was not observed in the HMBC, the strong

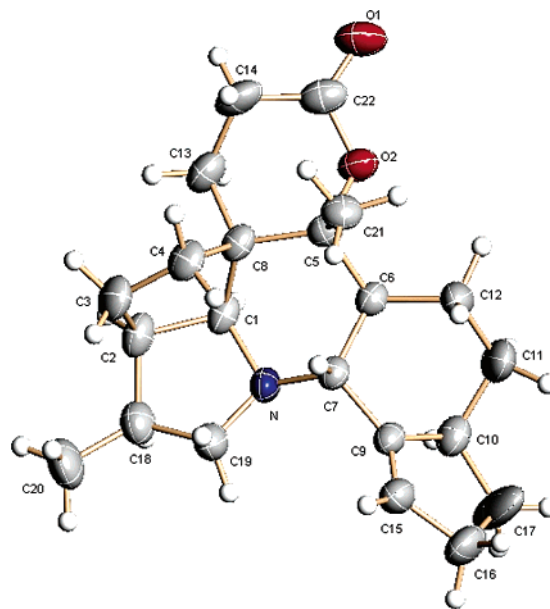


FIGURE 2. Single-crystal X-ray structure of deoxycalyciphylline B (**1**).

correlations between the H₂-11 and C-9 (J^{β}), and between the H₂-17 and C-9 (J^{β}) were indicative of the linkage of the C-10 and C-9. An oxygen-bearing quaternary carbon signal (sp^3) at δ 86.9 was assigned to the C-5 by the HMBC correlation with the H-6, and the only angular methyl was consequently attached to the C-5 by the strong HMBC correlation between the H₃-21 and C-5. The connectivity of two quaternary carbons C-5 and C-8 was tentatively linked by the HMBC cross-peaks between the H₂-4 and C-5 (J^{β}) and between the H₃-21 and C-8 (J^{β}). By default, the only ester bond could be assigned between C-5 and C-22 to form a six-membered lactone. The planar structure of deoxycalyciphylline B (**1**) was thus figured out.

The relative stereochemistry of **1** was fixed by NOESY spectrum (Figure 1B), in which the cross-peaks observed between the proton pairs of H-10/H-6, H-6/H-1, H-1/H-2, H-1/H-18, H-1/H₂-13, and H-2/H-18 indicated that the H-10, H-6, H-1, H-2, H-18, and CH₂-13 were in the α -orientation. The Me-21 and H-7 were assigned to be β -configuration judging from the NOESY correlations of H-4 β /H-7 and H₃-21/H-7. The A-ring was assigned as half-chair conformation judged by the presence of α -directed lactone and the NOESY correlation between H-1 α and H₂-13. The strong NOESY correlations of the proton pairs H-10/H-6 and H-6/H-1 clearly indicated that the both D- and E-rings took boat conformation. The five-membered B-, C-, and F-rings were tentatively furnished as envelope-conformation. A computer modeled 3D structure (Figure 4A) of **1** was generated by using the molecular modeling program CS Chem 3D Pro Version 6.0, using MM2 force field calculations for energy minimization. The relative stereochemistry and a favorable conformation of **1** offered by computer modeling were consistent with those of **1** assigned by NOESY spectrum.

The structure of **1** was ultimately confirmed by single crystal X-ray diffraction. The relative stereochemistry and conformation proposed by the NOESY spectrum and computer modeling were in good agreement with those established by the single crystal X-ray diffraction (Figure

TABLE 2. ^1H and ^{13}C NMR Data, HMBC, and NOESY Correlations of **2**

no.	δ_{C}^a	δ_{C}^b	δ_{H} , multi, J (Hz) (in CDCl_3)	HMBC (in CDCl_3) $\text{H} \rightarrow \text{C}$	NOESY (in CDCl_3) $\text{H} \rightarrow \text{H}$
1	73.2	76.1	3.17 (d, 4.6)		2, 6, 13 α , 18, 21
2	48.1	50.2	2.59 (m)	3, 5	1, 3 α , 3 β , 13 α , 13 β , 18, 20
3	22.4	24.0	α 1.87 (m) β 1.63 (m)	1, 2, 4, 5, 8, 18 1, 2, 4, 5, 8, 18	2, 4 α , 13 β , 18, 20 2, 4 β , 7, 19 β , 20
4	32.3	34.5	α 1.60 (m) β 2.01 (m)	1, 2, 3, 5, 8, 13 3, 5, 8, 13	3 α , 14 3 β , 7
5	85.7	87.6			
6	40.8	43.4	2.47 (dd, 15.0, 7.4)	5, 7, 11, 21	1, 10, 11 β , 12
7	58.6	61.4	3.78 (d, 7.2)	1, 5, 6, 9, 10, 12, 15, 19	3 β , 4 β , 11 β , 15, 19 β
8	48.9	50.6			
9	147.0	147.5			
10	41.9	44.8	2.59 (m)		6, 11 α , 12, 17 α , 21
11	30.5	32.1	α 1.90 (m) β 1.10 (ddd, 23.7, 12.2, 3.4)	6, 10, 12, 17 6, 10, 12, 17	10, 6, 7, 12, 17 β
12	23.5	26.0	1.75 (2H, m)	5, 7, 10, 11	6, 10, 11 β , 21
13	28.4	30.8	α 1.92 (m) β 1.63 (m)	1, 4, 8, 14 1, 4, 5, 8, 14, 21, 22	1, 2, 14, 21 2, 3 α , 14
14	26.3	27.5	2.75 (2H, m)	8, 13, 22	4 α , 13 α , 13 β , 21
15	127.0	130.1	5.59 (br.s)	7, 10, 16, 17	7, 16, 19 β
16	30.5	32.0	2.26 (2H, m)	10, 17	15, 17 α , 17 β
17	31.5	33.8	α 2.12 (m) β 1.45 (m)	9, 10, 11, 15, 16 10, 11, 16	10, 16 11 β , 16
18	35.5	37.4	2.39 (m)	19	1, 2, 3 α , 19 α , 20
19	60.0	61.9	α 3.08 (dd, 9.8, 7.5) β 2.51 (br.d, 10.0)	1, 7, 18, 20	18, 20 3 β , 7, 15, 20
20	14.3	15.1	1.03 (3H, d, 6.7)	2, 18, 19	2, 3 α , 3 β , 18, 19 α , 19 β
21	21.7	23.1	1.49 (3H, s)	5, 6, 8, 12	1, 10, 12, 13 α , 14
22	171.6	175.0			

^a Measured in CDCl_3 , ^b measured in CD_3OD .

2). The structure of deoxycalyciphylline B was thereby unambiguously elucidated as **1**.

Deoxyisocalyciphylline B (**2**) has the same molecular formula, $\text{C}_{22}\text{H}_{34}\text{NO}_2$, as **1**: HR EIMS m/z 341.2352 $[\text{M}]^+$ (calcd 341.2355). The IR absorption band at 1728 cm^{-1} indicated the presence of an ester carbonyl group. The ^1H NMR (Table 2) spectrum showed the presence of two methyls at δ 1.03 (3H, d, 6.8) and δ 1.49 (3H, s) and an olefinic proton signal at δ 5.59 (1H, br.s). The ^{13}C NMR (Table 2) pattern and EIMS fragmentation (see experimental) of **2** are very similar to those of **1**, suggesting that both compounds have a common structural feature. Analysis of HMQC, ^1H - ^1H COSY, and HMBC spectra (Table 2 and Figure 3A) allowed establishment of the planar structure of **2**, which is identical with that of **1**, suggesting that compound **2** is one of the stereoisomers of **1**.

In the ^{13}C NMR spectra measured in *d*-chloroform, the vinylic carbon signals assigned for the C-9 and C-15 showed broadening due likely to the presence of minor acids in the solvent. A solvent of d_4 -methanol was thus used for compound **2** to give a high quality ^{13}C NMR spectrum, in which the vinylic carbon signals of C-9 and C-15 become sharp (Table 2 and Supporting Information S11).

The NOESY (Table 2 and Figure 3B) interactions observed between the protons pairs of H-10/H-6, H-6/H-1, H-1/H-2, H-1/H-18, H-1/H-13 α (δ 1.92, m), and H-2/H-18 on the downside of the molecule clearly indicated that H-10, H-6, H-1, H-2, H-18, and CH_2 -13 were in α -orientation. The Me-21 was assigned as α -configuration judging from the strong correlation between H_3 -21 and H-1 in the NOESY spectrum. The H-7 correlating with the H-11 β , H-4 β , and H-19 β indicated that the H-7 was

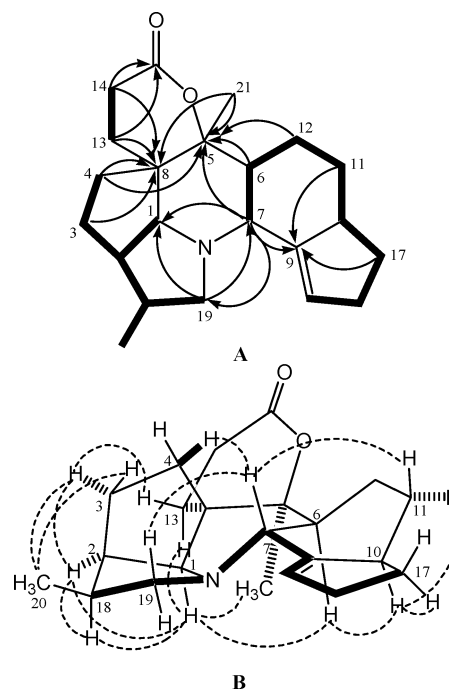


FIGURE 3. A: (—) ^1H - ^1H COSY of **2**; (○) selected HMBC Correlations of **2** ($\text{H} \rightarrow \text{C}$). B: (---) Key NOESY correlations of **2**.

β -orientated to form trans-fused D/E rings. The aforementioned NOESY correlations also outlined the conformation of **2**, in which from the A-ring to F-ring took half-chair, envelope, envelope, boat, boat, and envelope, respectively. The relative stereochemistry and conformation of **2** assigned by NOESY experiment were supported

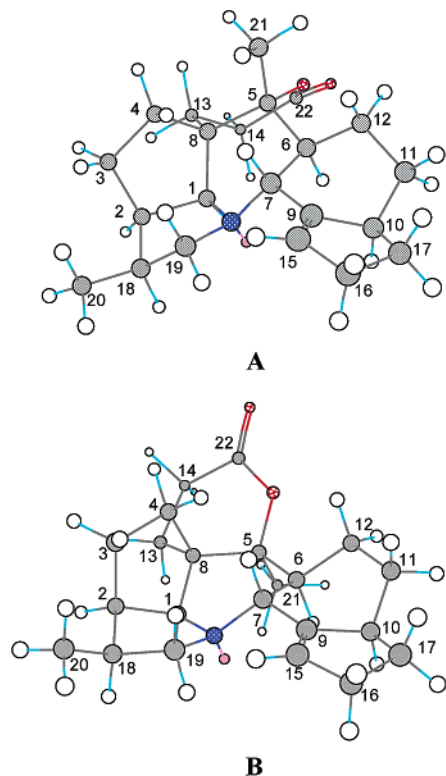


FIGURE 4. Stereoviews of **1** (A) and **2** (B) generated from computer modeling.

by the result of computer modeling (Figure 4B). The structure of deoxyisocalciphylline B was elucidated as **2**.

Compound **2** is an epimer of **1** differing at C-5, which allows the change of orientation of the A-ring in **2** (Figure 4). In consequence, the carbon signals of the A-ring (C-5, C-8, C-13, C-14, and C-22) and the adjoining carbon signals of C-4 and C-6 in **2** compared with those of **1** were obviously upfield-shifted, especially C-6 ($\Delta\delta_C -7.0$), C-8 ($\Delta\delta_C -3.2$), and C-4 ($\Delta\delta_C -4.2$). The former two were likely caused by the γ -gauche effects of C-22, and the later one probably resulted from the γ -gauche effects of both the C-14 and the oxygen in the ether bond. The downfield-shifted carbon signals of C-1 to C-3, C-9, C-7, C-10 to C-12 in compound **2** compared with those of **1** were considered to be caused by the deshielding effects of the carbonyl group. These deshielding effects seem to be distance-dependent, with the carbons that are closer to the carbonyl group in space showing larger chemical shift changes. The C-21 assigned for the methyl in compound **2** that was downfield-shifted ($\Delta\delta_C +1.9$) compared with that of **1** could also be demonstrated by the absence of γ -gauche effect of C-12 (in compound **1**, C-21 has γ -gauche effect with C-12). The slightly upfield-shifted carbon signals of C-15 to C-17 in the F-ring of **2** were also likely arisen by the γ -gauche effects of C-12 and C-11, which are closer to the F-ring in space compared with that of **1**.

Compound **3** was determined to be a quite recently isolated alkaloid calciphylline B^{1a} by 1D and 2D NMR spectra and was confirmed by the oxidation⁸ of **1** with

m-CPBA (*m*-chloroperbenzoic acid) to afford an oxidized product, which was consistent with the natural isolate **3** in both ¹H and ¹³C NMR data (Table 1). Most of the carbon signals of **3** were downfield-shifted compared with those of **1**, especially the C-7 ($\Delta\delta_C +12.0$), C-1 ($\Delta\delta_C +25.2$), and C-19 ($\Delta\delta_C +14.7$), and this was considered to be caused by the deshielding effect of the existence of *N*-oxide.⁸ The presence of γ -gauche effects of *N*-oxide on C-9 and C-6 resulted in obvious upfield shifts of the C-9 ($\Delta\delta_C -4.2$) and C-6 ($\Delta\delta_C -2.5$) compared with those of **1**.

Compound (**4**), namely, isocalciphylline B, was obtained as a new artifact by the oxidation⁸ of **2** with *m*-CPBA (*m*-chloroperbenzoic acid) at a mild condition.

Plausible Biogenesis of Compounds 1–3. It is of interest that the alkaloid compounds **1–3** isolated from this plant represent a unique fused hexacyclic frame. A plausible origin of these alkaloids (**1–3**) can be rationalized biogenetically as shown in the Scheme 1. The biogenetic origin of these alkaloids seems to be secodaphniphylline-type alkaloids, which underwent a C-7–C-10 bond cleavage to form a daphnilactone-B type intermediate (i). The intermediate (i) was transformed into an intermediate (ii) by a reasonable formation of the C-7–C-9 bond. The B–E rings were simultaneously constructed by the nucleophilic rearrangements of ii to form a key intermediate (iii), which further underwent an isomerizing procedure through an intermediate (iv) to produce a vital intermediate (v). The isomerization at C-6 offered the desired stereochemistry of a *trans*-fused D–E ring. The oxygen atom of the hydroxyl can attack at the C-5 from both the down- and up-side to generate deoxycalciphylline B (**1**) and deoxyisocalciphylline B (**2**), respectively. Deoxycalciphylline B (**1**) was then oxidized to produce calciphylline B (**3**).

Experimental Section

Plant Material. *Daphniphyllum subverticillatum* Merr. was collected in Hainan province of P. R. China and authenticated by Prof. Suhua Shi of Institute of Botany, School of Life Science, Zhongshan University of P. R. China. A voucher specimen has been deposited in the Herbarium of Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (accession number DS-2003-1Y).

Extraction and Isolation. The dry stem powder (1.6 Kg) of *D. subverticillatum* was percolated with 95% ethanol three times. After removal of the solvent under reduced pressure, the crude extract was dissolved in 1 L water to form a suspension and adjusted with 2 N H₂SO₄ to pH \approx 4. The acidic mixture was immediately defatted with ethyl acetate (500 mL \times 4), and the aqueous layer was basified with 5% Na₂CO₃ in water to pH \approx 10 and extracted with chloroform (300 mL \times 4) to obtain 320 mg crude alkaloids. The crude alkaloids were then subjected to a silica gel column eluted with chloroform/methanol/diethylamine (35/1/0.1, v/v/v) to yield deoxycalciphylline B (**1**, 120 mg), deoxyisocalciphylline B (**2**, 100 mg) and calciphylline B (**3**, 6 mg), sequentially.

Deoxycalciphylline B (1). Colorless quadrate crystals (acetone); mp 182 °C (dec); $[\alpha]_D^{20} = -96.0^\circ$ (*c* 1.17, CH₃OH); IR (KBr) ν_{\max} cm⁻¹ 3431 (very weak, water), 2933, 1738, 1456, 1271, 1140, 1076, 970; ¹H NMR see Table 1; ¹³C NMR see Table 1; EIMS 70 eV *m/z* (rel int) 341 [M]⁺ (84), 340 (66), 326 (13), 314 (21), 313 (100), 300 (19), 269 (11), 268 (19), 240 (14); HR-EIMS *m/z* 341.2346 (C₂₂H₃₄NO₂, calcd 341.2355).

Deoxyisocalciphylline B (2). Pale solid; $[\alpha]_D^{20} = -70.6^\circ$ (*c* 0.68, CH₃OH); mp 86–88 °C; IR (KBr) ν_{\max} cm⁻¹ 3431 (weak, water), 2949, 1728, 1456, 1389, 1256, 1157, 1080, 978; ¹H NMR see Table 2; ¹³C NMR see Table 2; EIMS 70 eV *m/z* (rel int.)

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341 [M]⁺(39), 340 (40), 326 (35), 314 (21), 313 (100), 298 (13); HR-EIMS *m/z* 341.2352 (C₂₂H₃₄NO₂, calcd 341.2355).

Oxidation of Compounds 1 and 2. A 10-mg portion of deoxycalyciphylline B (**1**) or deoxyisocalyciphylline B (**2**) was dissolved in CHCl₃ (1 mL), and then 12 mg *m*-CPBA was added at room temperature. After 3 h stirred, each reaction mixture was purified by silica gel column eluted with CHCl₃/CH₃OH/Et₂NH (20/1/0.1) to yield corresponding *N*-oxides, compounds **3** (9.8 mg) and **4** (9.0 mg).

Calyciphylline B (3). Colorless needles (acetone); [α]_D²⁰ = -70.7° (c 0.33, CH₃OH); mp 178 °C (dec); IR (KBr) ν_{max} cm⁻¹ 3427 (weak, water), 2928, 1736, 1456, 1273, 1128, 1082, 1047, 972, 769; ¹H NMR see Table 1; ¹³C NMR see Table 1; EIMS 70 eV *m/z* (rel int) 357 [M]⁺ (5.6), 342 (20), 341 (88), 340 (80), 326 (13), 313 (100), 300 (17), 268 (21), 267 (16), 266 (12), 240 (14); HR-EIMS *m/z* 357.2312 (C₂₂H₃₄NO₃, calcd 357.2304).

Isocalyciphylline B (4). Yellow gum; ¹H NMR (acetone-*d*₆) δ 5.77 (br.s, H-6), 3.98 (d, 7.0, H-8), 3.30 (br.s, H-12), 3.13 (m, H-17), 2.70 (m, H-27), 1.52 (3H, s, H₃-22), 1.03 (3H, d, 6.7, H₃-18).

Acknowledgment. The Financial support of the National Nature Science Foundation (30025044) of P. R. China and the foundation from the Ministry of Science and Technology (2002CB512807) of P. R. China are gratefully acknowledged. We thank Professor Suhua Shi for the collection and identification of the plant material.

Supporting Information Available: ¹H, ¹³C, and 2D NMR of deoxycalyciphylline B (**1**), deoxyisocalyciphylline B (**2**), and calyciphylline B (**3**), and ¹H NMR of isocalyciphylline B (**4**); CIF file, tables of crystal data and structure refinement, and list of bond lengths and angles from the X-ray crystallographic study of deoxycalyciphylline B (**1**). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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